Characterization of Diacylglycerylphosphocholine Molecular Species by FAB-CAD-MS/MS: A General Method Not Sensitive to the Nature of the Fatty Acyl Groups

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The use of negative ion mode fast-atom bombardment–collision-activated dissociation–tandem mass spectrometry (FAB-CAD-MS/MS) for diacylglycerylphosphocholine molecular species determinations was investigated for 24 naturally occurring and synthetic compounds. The previously proposed method of selecting $[M-15]^-$ as the parent ion and using the relative abundance of the carboxylate daughter ions to distinguish the positions of esterification was found to be unreliable in cases where the fatty acyl group at sn1 was much larger than that at sn2. The predicted greater abundance of the sn2 carboxylate daughter, relative to the sn1 carboxylate daughter, was also violated when polyunsaturated fatty acyl groups were esterified at sn2. In addition, several marginal cases were found where the ratio of intensities of the sn2/sn1 carboxylate daughters followed the expected pattern (sn2 > sn1) initially, but reversed over extended scanning time. The use of an alternative FAB-CAD-MS/MS method is proposed where the $[M-86]^-$ ion is selected as the precursor and the relative intensities of the daughters resulting from loss of the free fatty acids at sn1 and sn2 are determined. In every case examined to date, the ion formed by loss of the free acid from the sn2 position was always more abundant. Because the parent ion is equivalent to the phosphatidic acid ion, this technique should be equally applicable to all other phospholipid classes where this fragment ion is present in the spectrum. *(J Am Soc Mass Spectrom 1992, 3, 71–78)*

Fast atom bombardment–mass spectrometry (FAB-MS) and FAB–collision-activated dissociation–tandem mass spectrometry (FAB-CAD-MS/MS) techniques have been developed to characterize the polar head group and fatty acyl composition of a variety of glycerylphospholipid classes [1–6]. In the case of phosphatidylcholine (PC) derivatives (Figure 1), the three high mass ions found in the negative FAB-MS, $[M-15]^-$, $[M-60]^-$, and $[M-86]^-$, representing loss of a methyl group, a methyl plus dimethylamine, and a methyl plus *N,N*-dimethylvinylamine from the choline moiety, respectively (Figure 2a and b), have been used as parent ions in CAD-MS/MS experiments [1, 3, 4]. Jensen et al. [1] first pointed out that the intensity of the $[R^2COO]^-$ carboxylate daughter ion formed from the $[M-15]^-$ precursor is always greater than that of the $[R^1COO]^-$ ion, and that this could be used to establish the relative position of the two different fatty acyl moieties in a PC compound. It was reported that the carboxylate ion formed by cleavage of the fatty acyl group at the sn2 position was about two to three times [1, 3] more intense than that from the sn1 position. Jensen et al. [1], Münster and Budzikiewicz [3], and Hayashi et al. [4] also noted that similar ratios of carboxylate anions were found in the CAD-MS/MS spectra of the $[M-60]^-$ parent ion and that inverse ratios of the carboxylate daughter anions (i.e., $[R^1COO]^-/[R^2COO]^-$ or sn1/sn2 = 2–3) were observed when the $[M-86]^-$ ion was selected as the precursor. Although all three ions were stated to provide information concerning positional distribution, most emphasis has focused on CAD-MS/MS of the $[M-15]^-$ ion [1, 3]. These previous studies have all utilized commercially available samples of PC, most of which have fatty acyl groups of nearly equal chain lengths containing no more than three double bonds. Typically, in most naturally occurring PC compounds, the unsaturated fatty acyl

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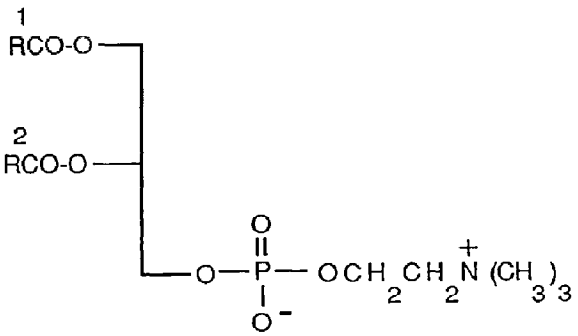


Figure 1. The structure of diacylglycerylphosphatidylcholine. The acyl groups designated R^1 and R^2 are attached at the sn1 and sn2 positions of the glycerol backbone, respectively.

group is located at the sn2 position, but a recent report described a series of compounds from rat brain that contain long-chain polyenoic acids esterified at the sn1 position [7]. Our interest in PCs containing polyunsaturated and long-chain fatty acyl groups led us to investigate a number of synthetically prepared compounds. Because several examples were encountered where the empirical rules described above were violated, we undertook a systematic study of the influence of fatty acyl group structures on the fragmentation pattern under FAB-CAD-MS/MS. In this report we discuss the scope and limitations of the empirical rule concerning carboxylate daughter ion abundances and utilize an alternative method for the determination of the relative position of fatty acyl groups in PC derivatives which is insensitive to the nature of the acyl moieties.

Experimental

Mass Spectrometry

Approximately 10 μg of phospholipid sample in a 1:1 $CHCl_3$-MeOH solution (1 μL) was mixed with 2 μL of triethanolamine matrix on the FAB probe tip. Ions were produced by bombardment with a beam of Xe atoms (6 kV) or Cs^+ ions (8 kV) in a JEOL USA (Peabody, MA) HX-110 double-focusing mass spectrometer (EB configuration) operating in the negative ion mode; no differences were observed in spectra acquired by neutral beam (Xe°) or charged cesium beam (Cs^+) bombardment. The accelerating voltage

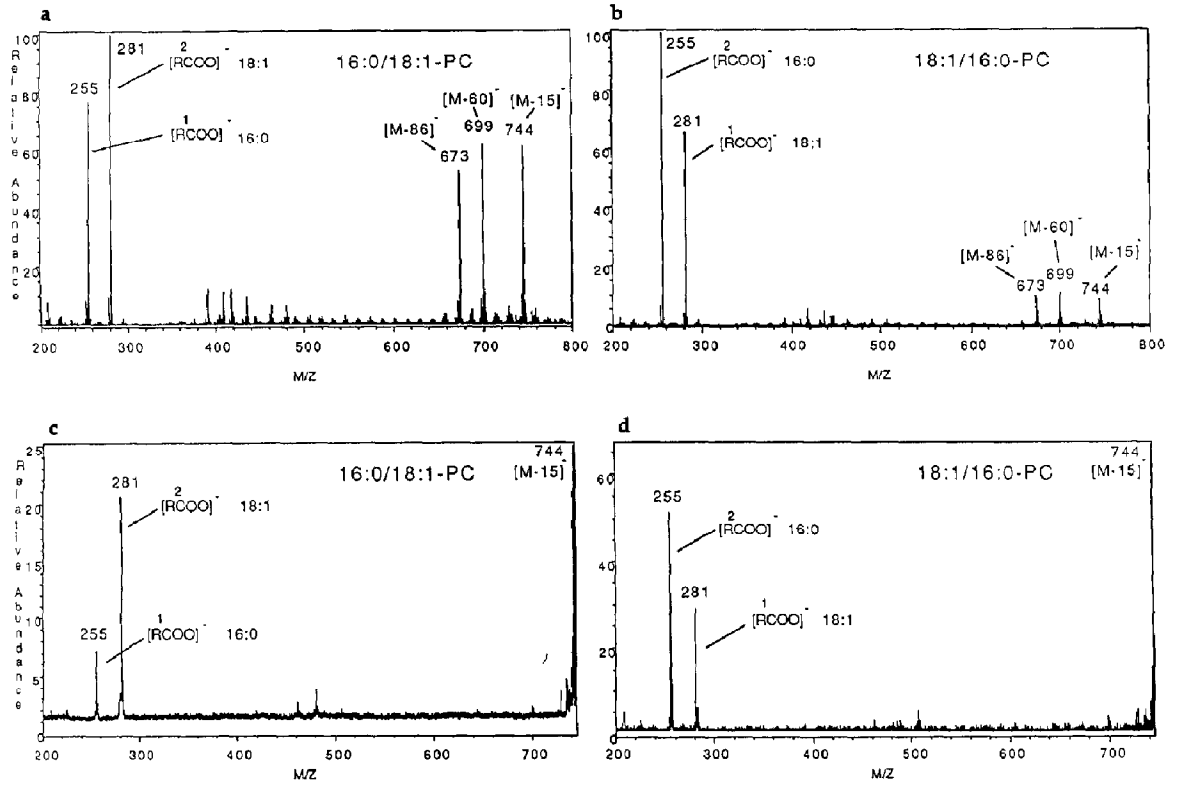


Figure 2. The negative FAB-MS of an isomeric pair of PC compounds, 16:0/18:1-PC, **9** (**a**), and 18:1/16:0-PC, **11** (**b**). The three high mass ions characteristic of PC derivatives, $[M-15]^-$, $[M-60]^-$, and $[M-86]^-$, are prominent. (**c**) and (**d**) show FAB-CAD-MS/MS of the $[M-15]^-$ ions of compounds **9** and **11**, respectively. The daughter ions abundances follow the empirical rule [1, 3, 4] where the sn2 carboxylate daughter ion is more intense than sn1 carboxylate daughter ion.

was 10 kV and the resolution was set at 1000. For CAD-MS/MS, helium was used as the collision gas in a cell located in the first field-free region. The helium pressure was adjusted to reduce the abundance of the parent ion by 50%. A JEOL DA-5000 data system generated linked scans at constant B/E. The instrument was scanned at a rate of 2 min from m/z 0–1500. Data presented were acquired in a single scan and were found to be reproducible.

Phosphatidylcholine

Compounds **9-12, 18, 23,** and **24** were obtained from Sigma Chemical Co. (St. Louis, MO). Other compounds were prepared synthetically, as described below.

Synthesis of Phosphatidylcholines

All PCs, except for those purchased commercially, were prepared by a modification of procedures reported in the literature [8–10]. An example for the preparation of 14:0/22:6-PC (**20**) is illustrated as follows: To a mixture of thoroughly dried 1-myristoyllysophosphatidylcholine (9.5 mg, 20.3 μmol), all-*cis*-(4,7,10,13,16,19)-docosahexaenoic acid (50 mg, 152 μmol) and *N,N*-dimethylaminopyridine (DMAP, 8 mg, 66 μmol) in 1 mL of chloroform (distilled over phosphorus pentoxide) was added to dicyclohexylcarbodiimide (DDCI, 31 mg, 152 μmol) in 500 μL of the same solvent. The reaction was allowed to proceed at ambient temperature under nitrogen, with vigorous stirring and protection from light, until thin-layer chromatography (TLC) showed a single spot of the diacylated product (6 h). The reaction was ended by adding 100 μL of methanol. After stirring for an additional 30 min, the precipitated dicyclohexylurea was filtered off. The filtrate was then applied to a 900-mg silica cartridge (Baxter Healthcare Corp., Muskegon, MI). The latter was washed with 40 mL of chloroform, 40 mL of chloroform-methanol (5:1 vol/vol) to remove the unreacted fatty acid and the catalyst, and then eluted with 4 × 10 mL of chloroform-methanol (1:3 vol/vol). The eluates were analyzed by TLC (silica gel, chloroform-methanol-water-ammonium hydroxide, 120:60:6:2 vol/vol) and visualized by Dittmer spray [11]. Fractions containing the diacylated product were combined and evaporated to dryness to yield 14 mg (88%) of the diacylated (14:0/22:6) PC as a colorless wax. The product was reconstituted in chloroform (1 mL), flushed with nitrogen, and stored at −80 °C before use. Alternatively, the coreaction product was separated and purified by column chromatography using 1:1 (wt/wt) iatrobead (Iatron Co., Tokyo)-silica gel or preparative TLC (see above). Overall yields for other compounds prepared in this manner were 44%–88%, based on lysophosphatidylcholine.

Evaluation of Positional Isomeric Purity of Phosphatidylcholine Samples

A solution of 25–50 μg of the PC in 500 μL of diethyl ether-methanol (20:1 vol/vol) was vortexed with 50 U of phospholipase A_2 (*Naja naja*, Sigma Chemical Co.) in 500 μL of 0.1 M Tris buffer containing 5 mM calcium chloride (pH 7.6) at ambient temperature for 3 h. The fatty acid thus liberated was taken up in diethyl ether (3 × 5 mL), which was dried over anhydrous sodium sulfate, filtered, and evaporated. An evaluation by negative ion FAB-MS or gas chromatography analysis (of the methyl esters) showed a positional purity ranging from 80% to 99% for selected PCs used in this study (see Tables 1 and 2).

Results and Discussion

Within the series of synthetic and naturally occurring compounds examined in this study, a number of PCs were found where the empirical rules [1, 3] for determining the relative positions of the fatty acyl groups were obeyed. An example using a pair of isomeric compounds, 16:0/18:1-PC, **9**, and 18:1/16:0-PC, **11**, is shown in Figure 2 to illustrate the general case. In the daughter ion spectra of the $[M-15]^-$ ion of each isomer, the sn2 carboxylate ions (m/z 281 and 255 for 18:1 and 16:0, respectively) are more intense (Figure 2c and d). Although the ratio of intensities of the sn2 to sn1 carboxylate ions approached 3:1 in the 16:0/18:1-PC isomer, **9** (Figure 2c), this ratio was < 2:1 in the 18:1/16:0-PC isomer, **11** (Figure 2d). Other compounds that produced daughter ion spectra fitting this general pattern are listed in Tables 1 and 2.

Generally, exceptions to the empirical rule based on carboxylate daughter ion intensities can be classified into two categories: those PC compounds with polyunsaturated (more than or equal to three or four double bonds) fatty acyl groups at the sn2 position and those PC compounds that contain a much shorter

Table 1. Effect of fatty acyl group chain length on daughter ion relative intensities: comparison of FAB-CAD-MS/MS of the $[M-15]^-$ and $[M-86]^-$ parent ions

	Compound[a]	Isomeric purity[b] (%)	$(M-15)^-$ parent $[R^2COO]^-/[R^1COO]^-$	$(M-86)^-$ parent $[M-86-R^2COOH]^-/[M-86-R^1COOH]^-$
1	14:0/8:0-PC		0.88	2.03
2	14:0/12:0-PC	94	1.73	2.42
3	14:0/16:0-PC		2.30	2.95
4	14:0/20:0-PC		3.00	1.93
5	16:0/5:0-PC		0.62	3.34
6	20:0/14:0-PC		0.97	1.38

[a] PC molecular species are designated throughout this article with the acyl groups at sn1 and sn2 listed in order, respectively (i.e., 14:0/20:0 = sn1/sn2 = R^1COO/R^2COO see figures).

[b] Isomeric purity was determined by phospholipase A_2 digestion and analysis of the liberated fatty acid by gas chromatography mass spectrometry as described in the Experimental section. The values are based upon the ratio of peak areas and are uncorrected for differences in response.

Table 2. Effect of fatty acyl unsaturation on daughter ion relative intensities: comparison of FAB-CAD-MS/MS of the $[M-15]^-$ and $[M-86]^-$ parent ions[a]

Compound		Isomeric purity (%)	$[M-15]^-$ parent $[R^2COO]^-/[R^1COO]^-$	$[M-86]^-$ parent $[M-86-R^2COOH]^-/[M-86-R^1COOH]^-$
7	14:0/18:1-PC		2.07	3.11
8	14:0/24:1-PC		3.29	3.40
9	16:0/18:1-PC		2.18	3.21
10	18:1/2:0-PC		0.12	1.71
11	18:1/16:0-PC	93	1.79	2.72
12	16:0/18:2-PC	94	1.88	3.13
13	14:0/18:3-PC		1.79	1.83
14	18:0/18:3-PC		1.26	1.98
15	20:0/18:3-PC	86	0.81	1.53
16	14:0/20:3-PC		1.37	2.44
17	20:0/20:3-PC		0.55	2.00
18	18:0/20:4-PC	95	1.88	5.33
19	20:0/20:4-PC		1.12	3.50
20	14:0/22:6-PC	90	0.78	4.18
21	18:0/22:6-PC		0.41	1.64
22	20:0/22:6-PC	96	0.44	1.50
23	16:0/pyr-PC	80	3.52	2.58
24	Pyr/16:0-PC	99	1.38	4.42

[a]Unsaturated fatty acyl groups were designated by the convention, carbon number: double bond number (i.e., 18:1 (oleoyl), 24:1 (nervonoyl), 18:2 (linoleoyl), 18.3 (linolenoyl), 20:3 (all-*cis*-11,14,17-eicosatrienoyl), 20:4 arachidonoyl, 22:6 (all-*cis*-4,7,10,13,16,19-docosahexaenoyl)). Pyr, [(pyren-1-yl)hexanoyl].

(≥ 6 U of methylene) fatty acyl group at sn2 than that at sn1. In addition, several marginal cases were found where, although the sn2 carboxylate daughter ion from the $[M-15]^-$ parent was initially more abundant than the sn1 carboxylate ion, the ratio of intensities reversed during prolonged scanning. Each of these exceptions will be discussed in turn.

Saturated Diacyl Phosphatidylcholine Compounds

The CAD-MS/MS spectra of the $[M-15]^-$ ion of six saturated diacyl PCs, four in a homologous series of compounds containing a 1-myristoyl function, were acquired. In cases where a longer-chain acyl function resided at sn2, the carboxylate daughter ion from the fatty acyl group at this position was substantially more intense than that from the sn1 position (Table 1). In the two compounds analyzed (14:0/16:0-PC and 14:0/20:0-PC), ratios of the two carboxylate peak intensities (sn2/sn1) were greater than 2.0, in accord with previous reports [1, 3]. This pattern did not hold, however, in the samples analyzed that contained a shorter acyl function at the sn2 position. A strong trend in the ratio of carboxylate daughter ions of $[M-15]^-$ was observed when the length of the fatty acyl group at sn1 relative to that at sn2 was increased; as the fatty acyl group at sn2 decreased in size, the ratio of the sn2:sn1 carboxylate daughter ion intensities $[R^2COO]^-/[R^1COO]^-$ also decreased. In the homologous series of compounds containing a myristoyl group at sn1, and saturated fatty acyl groups at sn2 with a small difference in chain length between the two acyl groups (e.g., 14:0/12:0-PC, **2**), the sn2 carboxylate daughter was more intense, as predicted (for **2** the ratio is 1.73). In contrast, the expected ratio of intensities no longer held in compounds where the differences in the acyl chain lengths were larger (e.g., 14:0/8:0-PC, **1**, Figure 3). Several other compounds with saturated sn1 acyl function and with significantly shorter acyl groups at sn2 (16:0/5:0-PC, **5** and 20:0/14:0-PC, **6**) exhibited similar daughter ion spectra, with much greater intensity of the sn1 carboxylate ion relative to the sn2 carboxylate (Table 1). The effect of differences in carboxylate chain length was most pronounced in a compound (**10**) that was acetylated at the sn2 position (Table 2). The observation that chain length differences could affect carboxylate daughter abundances was mentioned previously for PCs containing an acetoxy function at sn2 [3].

Phosphatidylcholine Compounds Containing an Unsaturated Fatty Acyl Group at the sn2 Position

Eighteen synthesized or commercially available PC derivatives containing unsaturated fatty acyl groups at the sn2 and sn1 positions were analyzed. In the FAB-CAD-MS/MS spectra of the $[M-15]^-$ ion of the compounds containing one or two double bonds (**7–9, 11, 12**), the carboxylate daughter ion formed by cleavage of the sn2 fatty acyl moiety was more intense than that of the sn1 acyl function (Table 2). However, the ratio of intensities of these two daughter ions (sn2 carboxylate/sn1 carboxylate) tended to decrease with increasing number of double bonds (Table 2). This trend was apparent in a number of homologous series including 1-myristoyl-PC (**7, 13, 16, and 20**), 1-stearoyl-PC (**14, 18, and 21**), and 1-arachidoyl-PC (**15, 17, 19,** and **22**) (see Table 2). When the number of double bonds in the sn2 fatty acyl group was four or

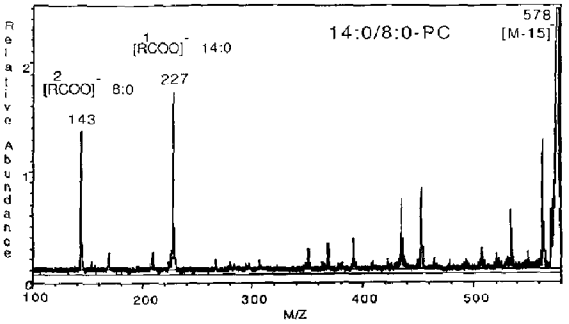


Figure 3. FAB-CAD-MS/MS of the $[M-15]^-$ ion of 14:0/8:0-PC (**1**). The carboxylate ion formed by cleavage of the sn2 octanoyl acyl group (m/z 143) is less intense than the sn1 myristate ion (m/z 227), counter to expectations [1, 3, 4].

greater, the ratio of intensities of the carboxylate daughter ions of the $[M-15]^-$ parent deviated from the expected pattern in all but one example (18:0/20:4-PC (**18**), ratio 1.88) (see Table 2). The significantly larger peak for the sn1 carboxylate ion relative to the sn2 carboxylate was particularly apparent in compounds containing docosahexenoic acyl functions at sn2 (Table 2, **20–22**) where the ratio (sn2/sn1) was much less than 1.0 (Figure 4). The MS/MS spectrum of the $[M-15]^-$ ion of another compound (20:0/18:3-PC, **15**) with a different sn2 group displays the same pattern (Figure 5). Here both sn2 acyl group unsaturation and chain length differences between the two acyl groups may be influential.

Within the homologous series having a fixed polyunsaturated fatty acyl group at sn2, and various fatty acyl groups at the sn1 position, the ratio of sn2/sn1 carboxylate daughters of $[M-15]^-$ decreased with increasing carbon number of the sn1 acyl group (Table 2, **13–15**; and **16–19**; and **20–22**).

Changes in Carboxylate Daughter Ion Abundance During Extended Scanning

Several examples (**16**, **19**, and **24**) were encountered where the carboxylate daughter ion abundances changed over time during FAB-CAD-MS/MS scanning. Two of these compounds, 14:0/20:3-PC (**16**) and 20:0/20:4-PC (**19**), displayed more intense sn2 carboxylate daughter ions in the spectra acquired during the first scan (ca. 1 min 30 s). However, by the third scan (ca. 3–4 min) the sn2 carboxylate daughter ion was less intense than the sn1 carboxylate (Figure 6). Similar differences in fragment ion abundances during prolonged scanning have been ascribed to changes in

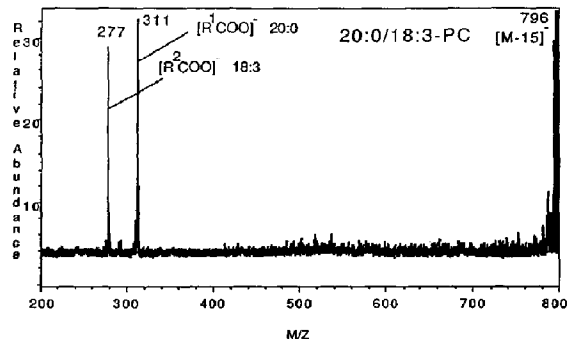
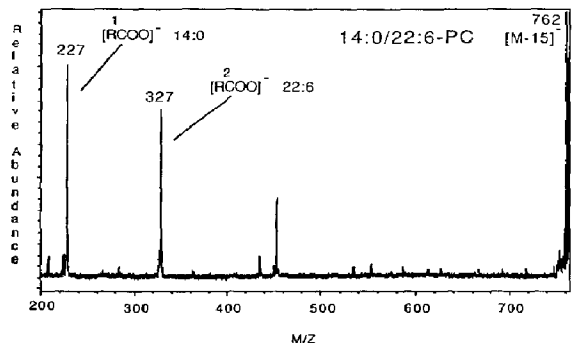


Figure 5. FAB-CAD-MS/MS of the $[M-15]^-$ ion of 20:0/18:3-PC (**15**).



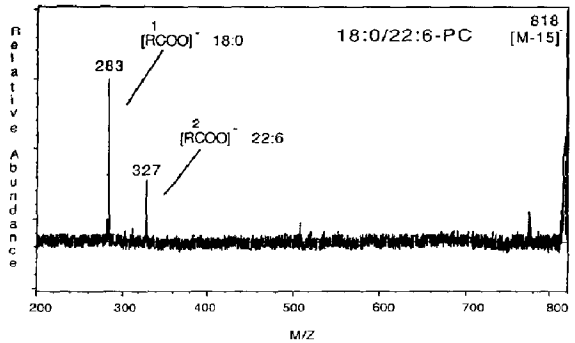
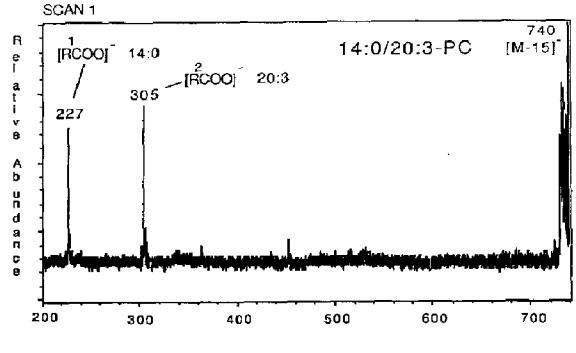


Figure 4. FAB-CAD-MS/MS of the $[M-15]^-$ ion of 14:0/22:6 PC (**20**) and 18:0/22:6 (**21**). The carboxylate anions from the polyunsaturated fatty acyl group (22:6) at the sn2 position are less intense than the carboxylates from the sn1 position.



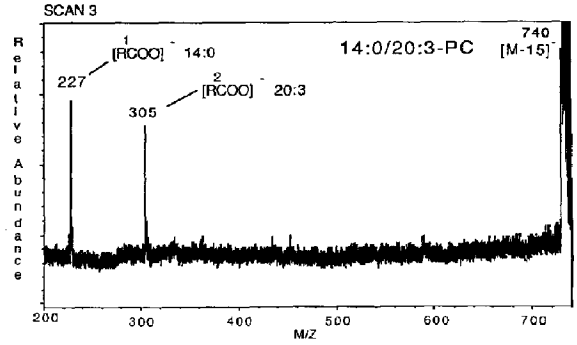


Figure 6. FAB-CAD-MS/MS of the $[M-15]^-$ ion of 14:0/20:3-PC (**16**) shows the effect of scanning over an extended period (ca. 5 min). The carboxylate daughter ion ratios were observed to change from the first scan to the third scan (~ 3–4 min).

the internal energy of the parent ion owing to changing solvation of the desorbed molecules by the FAB matrix over time [12].

An Alternative Approach for the Determination of Acyl Group Positions in Phosphatidylcholines

Each of the exceptions described above demonstrates that the relative intensity of the carboxylate daughter ions from the $[M-15]^-$ parent of diacyl PC derivatives cannot always be relied upon for unambiguous determination of the relative position of the two acyl groups. However, the use of an alternative FAB-CAD-MS/MS experiment does permit acyl position assignments to be made with confidence, even for the exceptional cases discussed. If $[M-86]^-$ (PC minus choline, equivalent to the analogous phosphatidic acid) is used as the parent ion, a significantly different daughter ion spectrum is obtained [1, 3, 4]. Although the two carboxylate daughter ions are still prominent, their ratio of intensities is usually reversed $[R^1COO]^- > [R^2COO]^-$ (i.e., sn1 > sn2) [1]. Yet there are compounds where this prediction is also violated. For example, FAB-CAD-MS/MS of the $[M-86]^-$ ion of both 14:0/20:0-PC (**4**) and 14:0/24:1-PC (**8**) demonstrated that the carboxylate daughter ion from the sn1 position is less intense than that from the sn2 position (data not shown).

Four daughter ions arising from loss of the two free fatty acids (i.e., $[M-86-RCOOH]^-$) and their ketene analogs (i.e., $[M-86-R=C=O]^-$) are significant peaks in the spectrum. It was earlier reported that loss of the free acid from the sn2 position occurs more readily from CAD-MS/MS of the $[M-86]^-$ parent [1, 4]. We have now systematically investigated the utility of daughter ion abundances formed by the loss of the sn1 and sn2 free fatty acids from the $[M-86]^-$ parent ion of PC derivatives. In every case so far examined the loss of the free fatty acid from the sn2 position is always more abundant than that from the sn1 position (Tables 1 and 2). For example, in the PC (**20**) containing a 14:0 fatty acyl function at sn1 and a 22:6 fatty acyl function at sn2, the loss of 22:6 as the free fatty acid from the $[M-86]^-$ parent is significantly more intense than the loss of myristic acid (Figure 7). This is in marked contrast to the violation of the expectation of carboxylate daughter ion abundances from the $[M-15]^-$ parent of this compound (Figure 4). Further, chain length differences do not appear to influence the tendency of $[M-86-R^2COOH]^-$ to dominate in the daughter ion spectra (Table 1). As shown in Figure 8, the use of this technique would reliably predict the relative acyl group positions in 1, 14:0/8:0-PC (cf. Figure 3). An added advantage is that the $[M-86-RCOOH]^-$ daughter ion intensities are stable over extended scanning periods. In contrast to the change in the relative abundances of the carboxylate daughters of the $[M-15]^-$ ion of **16** (Figure 6), the relative intensities of the two lysophosphatidic acid

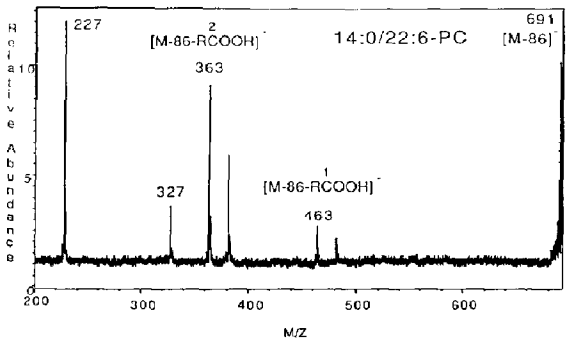


Figure 7. FAB-CAD-MS/MS of the $[M-86]^-$ ion of 14:0/22:6-PC (**21**). The daughter ion formed by loss of the free fatty acid from the sn2 position is more intense than the daughter formed by loss of that from the sn1 position.

daughter ions of the $[M-86]^-$ ion of this compound appear to be consistent over time for the compounds examined so far (see Figure 9).

The relative intensities of the daughter ions of $[M-86]^-$ formed by loss of the neutral fatty acids also appear to be less sensitive to collision conditions than the relative abundances of the carboxylate daughters of this parent. We have observed that the metastable decomposition spectrum of the $[M-86]^-$ ion shows preferential neutral loss of the fatty acid from the sn2 position (e.g., 14:0/18:3-PC, **12**, data not shown). In contrast, while the sn1 carboxylate daughter is predicted to be more abundant than the sn2 carboxylate daughter from $[M-86]^-$ [1, 4], in some selected cases under metastable conditions (e.g., **12**) the sn2 carboxylate daughter is more abundant (note, however, that in the high energy CAD-MS/MS of the $[M-86]^-$ ion of **12**, the sn1 carboxylate is relatively more intense than the sn2 carboxylate, as expected from previous reports [1, 4]).

The metastable decomposition results imply that under low energy CAD conditions, daughters of $[M-86]^-$ formed by loss of the sn2 free fatty acids, $[M-86-R^2COOH]^-$, would still be more abundant. This was

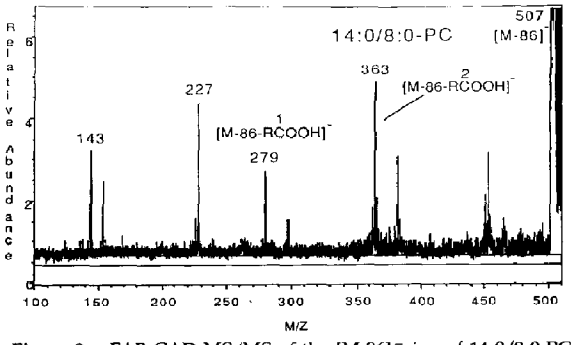


Figure 8. FAB-CAD-MS/MS of the $[M-86]^-$ ion of 14:0/8:0-PC (**1**).

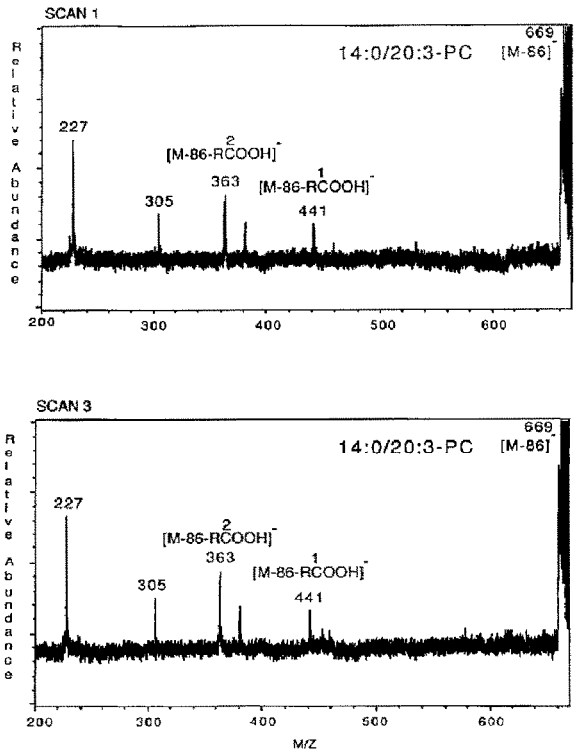


Figure 9. FAB-CAD-MS/MS of the $[M-86]^-$ of 14:0/20:3-PC (**16**) demonstrates that the ratio of daughter ions formed by loss of the free fatty acids are not affected by extended scanning (cf. Figure 6).

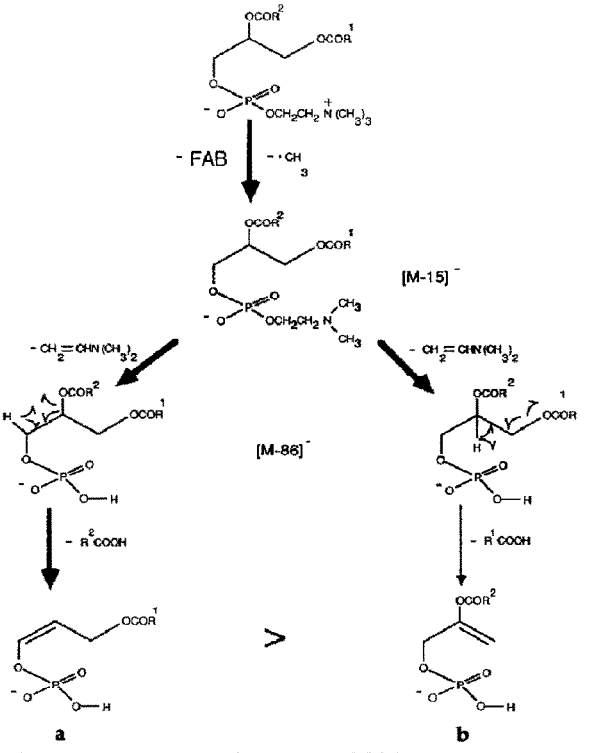


Figure 10. Formation of the $[M-86-RCOOH]^-$ ion showing preference for the loss of the sn2 fatty acyl group (**A**) over the sn1 fatty acyl group (**B**). Note that it has been demonstrated that both the $[M-15]^-$ and $[M-86]^-$ ions are formed indirectly from a matrix adduct to the PC compound, rather than directly from fragmentation of the PC [4, 13].

confirmed by conducting this daughter ion experiment with a number of different compounds on a triple quadrupole mass spectrometer (0.1 mtorr argon, collision energy 5–30 eV) [M. Cole, personal communication]. Thus, this alternative approach appears to be applicable to a wide variety of PC compounds, using different instrumental parameters. Further studies of PC fragmentation under low energy CAD conditions are now underway.

An explanation for the relative abundance of daughters formed by loss of the free fatty acids may involve the stability of the product ion formed. A proposed mechanism for the formation of the daughter ion is shown in Figure 10. The elimination from the glyceryl moiety of the secondary ester function is probably more favored than the elimination of the primary ester because the latter results in the formation of an isolated terminal double bond (Figure 10, structure **B**, $[M-86-R^1COOH]^-$). The conjugation with the phosphate moiety of the internal double bond formed by the alternative pathway (Figure 10, structure **A**, $[M-86-R^2COOH]^-$) may provide some added stability relative to structure **B**. Because the fatty acyl group at sn2 is expelled as the free acid, subsequent fragmentation or reactions of the fatty acid group do not influence the abundances of the lysophosphatidic acid daughter ions. These subsequent reactions may account for differences in carboxylate daughter ion abundances in PCs containing polyunsaturated fatty acyl functions. As shown in Figure 11, equimolar quantities of saturated and unsaturated fatty acids may not display $[M-H]^-$ ions in equal abundance.

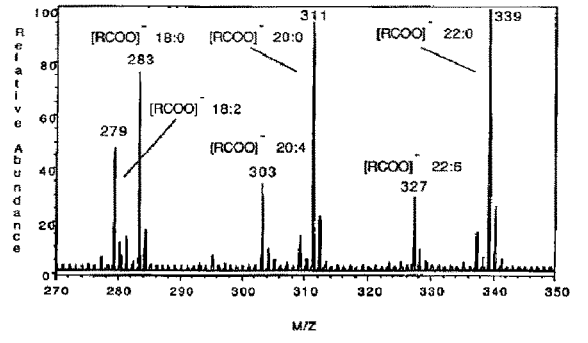


Figure 11. Negative FAB-MS of an equimolar mixture of fatty acids shows the influence of structure on carboxylate ion abundance.

The discrimination in favor of the saturated [14] and long-chain carboxylate anions may provide additional explanation as to why the carboxylate daughters cannot be used reliably to determine PC fatty acyl group positions.

The use of the relative intensities of daughters formed by loss of RCOOH from the $[M-86]^-$ parent ion of diacyl PC compounds suggests that this CAD-MS/MS approach may have general applicability to other classes of diacylglycerylphospholipids (PL). Because this ion is equivalent to phosphatidic acid, the same ion found in other PL classes can be subjected to CAD-MS/MS analysis. Preliminary studies with diacylglycerylphosphoserine and diacylglycerylphosphoinositol compounds have demonstrated that the free fatty acid from the sn2 position is also preferentially lost from the $[M-88]^-$ and $[M-162]^-$ ions, respectively (equivalent to the phosphatidic acid), found in the spectra of these compounds.

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